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Abstract

Backgrounds and aims:
The functioning of temperate forests may change dramatically in the future due to more extreme precipitation events. In contrast to drought effects, little is known about the reaction of soil fungi to rewatering. We studied soil fungal communities and soil enzymatic activities over a period of 3 months following rewatering after 5 years of experimental drought.
Results:
The most pronounced changes compared to the drought phase occurred early after rewatering in the beech root zone and were mainly attributed to litter decomposers. In the spruce zone, the relative abundance of ectomycorrhizal fungi (ECMf) was lower during the initial phase of response to rewatering but approached control levels after 3 months. The previous drought treatment was influencing the structure of the saprotrophic fungal community (SAPf) more than that of the ECMf community during rewatering. The composition of the SAPf community was associated with changes in nitrogen (mineral nitrogen: control 2.86, rewatering = 1.53), while that of the ECMf community was associated with the soil water content (control = 26%, and rewatering = 22%). Soil enzyme activities were positively correlated with the diversity and composition of SAPf communities, especially in previously drought-

treated plots. In beech and mixed root zones, plant cell wall-degrading enzyme activities were elevated in rewatered plots compared with control plots, while in spruce, only cellobiohydrolase and β-glucosidase were elevated.
Conclusion:
Structural changes within SAPf communities associated with nitrogen dynamics correlated with enzymatic activity in response to rewatering. A low responsiveness of fungal community composition in the mixed root zone suggests its buffering capacity against fluctuating soil moisture conditions.

Keywords (separated by '-') Forest soil fungi - Soil enzyme activities - Norway spruce - European beech - Mixed interaction - Experimental drought - Rewatering

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RESEARCH ARTICLE

Effects of rewatering on soil fungi and soil enzymes in a spruce-beech forest after a 5-year experimental drought

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beech and mixed root zones, plant cell wall-degrading enzyme activities were elevated in rewatered plots compared with control plots, while in spruce, only cellobiohydrolase and β-glucosidase were elevated.

Conclusion Structural changes within SAPf communities associated with nitrogen dynamics correlated with enzymatic activity in response to rewatering. A low responsiveness of fungal community composition in the mixed root zone suggests its buffering capacity against fluctuating soil moisture conditions.

Keywords Forest soil fungi · Soil enzyme activities · Norway spruce · European beech · Mixed interaction · Experimental drought · Rewatering

Introduction

Many forest ecosystems in Europe are at risk because of the predicted highly variable precipitation and temperature regimes (Sherwood and Fu 2014; IPCC 2021). Few studies on forest tree drought have included the recovery process after drought and the mechanisms employed by different tree species (Arend et al. 2022; Hikino et al. 2022; Grams et al. 2021). The two dominant forest tree species in Central Europe, Norway spruce (*Picea abies* [L.] Karst) and European beech (*Fagus sylvatica* [L.]) are considered vulnerable to drought (Pretzsch et al. 2014, 2020; Leuschner 2020). However, both tree species often grow better in mixed stands than in monocultures (cf. Pretzsch et al. 2020). In the case of spruce and beech, positive mixture effects have been attributed to the overall beneficial trait complementarity of both tree species, such as differences in the seasonality of water use (Allen et al. 2019), litter types (Berger and Berger 2012), rooting depths (Zapater et al. 2011), and fine root growth (Nikolova et al. 2020). A higher diversity of niches resulting from this complex trait diversity also influences the composition and functional roles of soil fungal communities (Asplund et al. 2018, 2019).

Under soil drought, the relative abundance of fungal functional groups is changed (Ekblad et al. 2013), and soil saprotrophic fungi (SAPf) are more affected than ectomycorrhizal fungi (ECMf) (Castaño et al. 2018). SAPf are particularly exposed to changes in the soil physicochemical environment, and their performance under drought conditions depends on the specific response of fungal species (Schimel et al. 2007). In addition to environmental factors, the composition of forest soil fungal communities is largely driven by tree species (Tedersoo et al. 2016). Thus, soil fungal community composition may be indirectly affected by tree species-specific reactions to soil drought (Buscardo et al. 2021; Baldrian et al. 2023), i.e., adapted root growth (Nikolova 2008; Nikolova et al. 2020), changes in root exudation (Brunn et al. 2022), regulation of water use (isohydric vs. anisohydric, Hesse et al. 2022; Ulrich and Grossiord 2023), hydraulic redistribution (Pretzsch et al. 2014; Zapater et al. 2011), and increased amounts of root and leaf litter (Landesman and Dighton 2011). In contrast to predominantly soil living and litter associated SAPf, ECMf are physically and physiologically connected to their host tree and thus particularly depend on the tree species-specific fine root reaction to drought (Lehto and Zwiazek 2011). This may also apply for root endophytic fungi and saprotophic fungi with a secondary root associated life style. ECMf form mutualistic symbioses with tree fine roots, including species of Pinaceae and Fagales (Smith and Read 2010). ECMf are taxonomically diverse (Tedersoo et al. 2010), but can be classified morphologically into different exploration types as long-, medium-, contact types (Agerer 2001). This classification accounts for the different extents the soil volume can be exploited by mycorrhizal roots through their extramatrical hyphae at different distances depending on the exploration type (Agerer 2001). It is discussed that long-distance exploration type ECMf have access to root-unaccessible water and thus support the tree drought survival (Lamhamedi et al. 1992). However, the observation of an ambiguous trend or even an increase in exploration types characterized by a lower biomass of extramatrical mycelium in the soil under drought conditions (Barnes et al. 2018; Castaño et al. 2018, Köhler et al. 2018) may indicate that an increase in the abundance of ECMf forming the long-distance exploration type under drought conditions may occur with a concomitant increase in photosynthetic efficiency, allowing the maintenance of mycelium that requires an increased supply of C (Castaño et al. 2023)..

Spruce and beech trees have been studied for their fine root growth under single and repeated droughts (Nikolova 2008; Nikolova et al. 2020; Zwetsloot and

Journal : Medium 11104 | Article No : 6564 | Pages : 22 | MS Code : 6564 | Dispatch : 23-2-2024

Bauerle 2021). Under lasting severe drought, spruce fine roots become suberized and stay alive in a state of dormancy, while beech fine roots are subject to constant renewal under drought but with a short life span and fast turnover (Nikolova 2008; Nikolova et al. 2020). This is thought to allow ECMf to colonize newly formed beech fine roots, while suberization of spruce fine roots and a very limited formation of new fine roots over longer drought periods may hinder recolonization (Sharda and Koide 2008). Upon rewatering, a faster regeneration of ectomycorrhizae with a smaller extramatrical mycelium (Tedersoo and Smith 2013) may also influence the composition of ECMf communities in soil. However, ECMf communities on fine roots showed little response closely associated with tree species-specific response patterns related to root survival and recovery (Danzberger et al. 2023).

Drought causes changes in the physical and chemical conditions in soils and strongly reduces soil microbial activities (Castaño et al. 2018) resulting in low soil respiration and decreased extracellular enzyme activities (Baldrian et al. 2010; Brockett et al. 2012). This leads to an accumulation of litter (Landesman and Dighton 2011) and nutrients, e.g., nitrogen, in the soil (Schimel et al. 2007), which in turn further influence fungal community composition (Högberg et al. 2003). Soil fungal communities play an integral functional role in forest soil nutrient cycling (Lindahl and Tunlid 2015), and SAPf are the main decomposers of dead organic materials such as leaf litter and wood in forest soils (Talbot et al. 2013; Asplund et al. 2018). SAPf are characterized by their high genetic potential for enzymatic decomposition (Baldrian 2017) in contrast to ECMf (Lindahl and Tunlid 2015). Enzymes, being responsible for acquisition of the three main nutrients carbon, nitrogen, and phosphorus respond differently to the same degree of soil moisture reduction (Sardans and Peñuelas 2005). The activity of soil enzymes, even of the same enzyme, in response to precipiation can be significantly modified by the plant species (Kreyling et al. 2008; Zhou et al. 2013). The composition and functional changes of soil microbial communities as well as activities of extracellular soil enzymes may be driven by the changes in soil moisture, microclimate and plant root exudates (Puissant et al. 2015). Thus, soil biological feedback is dependent also on the reaction of plants upon drought variables.

Upon rewatering, physicochemical conditions in soils change abruptly with different reaction patterns of soil microbial communities (Fierer et al. 2021). While soil bacterial biomass increased within hours, soil fungal biomass did not change over weeks in a pine forest (Landesman and Dighton 2011). In a recent study, Joseph et al. (2020) showed that even small additions of water in a dry Scots pine forest led to a regain of rhizosphere microbial activity. Although the structural and functional dynamics of changes in soil fungal communities in response to rewatering have been poorly understood, there has been even less understanding of how this is related to soil enzyme activity, particularly upon influence of different tree species. At the Kranzberg roof (KROOF) experimental forest site, controlled rewatering after 5 years of summer rain exclusion revealed a faster recovery of beech than spruce (Grams et al. 2021).

Here, we focused on the dynamics of soil fungal communities during the 3-month period after controlled rewatering in the KROOF experiment. We examined how changes in soil abiotic condition (the soil water and nitrogen content) may drive the composition of the soil fungal community and connected with them the soil enzymes involved in nutrient cycling in monospecific and two species mixed root zones (beech and spruce) to each other. Because of the stronger soil influence on SAPf than ECMf, we hypothesized the following:

H1: SAPf communities are more responsive than ECMf communities to rewatering due to a change in abiotic soil conditions that is faster than the speed of root regeneration; rewatering will favor contact exploration type ECMf.

H2: The response of soil fungal communities will be modified by the root interaction zones; in particular, fungal community composition (taxonomic and functional groups) will more quickly resemble controls in the mixed root zone than in the monospecific root zones.

H3: Changes in soil enzyme activities reflect changes in the SAPf community composition and are more pronounced in monospecific zones than in mixed zones due to greater changes in abiotic factors.

Journal : Medium 11104 | Article No : 6564 | Pages : 22 | MS Code : 6564 | Dispatch : 23-2-2024

Material and methods

Research site and sampling

The experimental "Kranzberg Forest" site is located in Southern Germany (11°39,042″E, 48°25012″N; 490 m a.s.l.) with an average annual precipitation of 750–800 mm and a mean annual air temperature of 7.8 °C (1971–2000) (Pretzsch et al. 2014). The experiment was set up in a mature stand with Norway spruce (*P. abies* (L.) Karst.) and European beech (*F. sylvatica* L.) grown in luvisol originating from loess over Tertiary sediments (for more details, see Grams et al. 2021). In 2011, 12 plots with a size of 111–199 m^2 were established. A thick plastic tarp was installed in 1 m deep trenches to avoid lateral water flow (Grams et al. 2021). Each plot contained at least three beech and three spruce trees, leading to three tree root zones: mainly intraspecific root contact with beech or spruce and interspecific root contact of both tree species (Mix). Six plots served as controls receiving ambient precipitation, and six plots were assigned to throughfall exclusion using retractable roofs below the canopy to exclude precipitation during the vegetation period (March-November) from 2014–2018. Control and rewatering plots were arranged pairwise next to each other across the experimental site. Temperature on site was measured every 10 min at 2 m height, and volumetric soil water content was recorded by time-domain reflectometer sensors on each plot and in each tree root zone (Grams et al. 2021).

In 2015, two rewatering plots and the neighboring control plots were excluded from the study because spruces were felled after bark beetle infestation. In early summer 2019, the remaining four rewatering plots were watered by drip irrigation to attain the soil water content of the control plots ("rewatering") (Grams et al. 2021). Watering of the plots was performed in three campaigns within 4 weeks, as the intensive sampling and sample processing activities did not allow to water all plots at the same time. In each campaign, soil samples were collected 7 days (d) before (-7 d) and after irrigation at 7 d, 18 d, 42 d, and 84 d. On each sampling date, 10 soil cores (diameter 1.4 cm, 25 cm depth) were taken from each tree root zone on each plot, and the upper organic-rich layer, visible by a dark color, with a depth of 0–10 cm. was pooled. The samples were homogenized at the sampling site. The soil cores were pooled before filled in the bag and then the soil subsample were taken for the further analysis. Subsamples designated for the enzyme activity test and DNA analyses were retrieved from each such soil sample, avoiding roots and particles > 2 mm in diameter. These soil samples were placed on dry ice within 30 min and frozen at -80 °C until further processing. In addition, 3 g and 5 g of fresh soil were weighed into 50 mL plastic tubes to determine the gravimetric water content and nitrogen content, respectively. In summary, the sampling included 2 treatments × 4 plots × 3 tree root zones × 5 time points, resulting in a total of 120 samples, giving 4 replicates of each treatment.

Soil enzyme analysis

Soil samples were thawed and allowed to adapt for 1 day at 6 °C prior to analyses. A total of 400 mg of soil was mixed with 40 ml of distilled water and shaken vigorously first for 10 s by hand and then for 15 min on an overhead shaker at room temperature at 100 rpm. The soil suspension was ultra-sonicated in an ice water bath for 3 min and filtered through a 90 µm nylon mesh to remove coarse particles, and filtrates were immediately used for enzyme assays. 3 g of the same soil sample were used to determine the soil dry matter in each sample.

Soil enzyme activities were determined using methylumbelliferone (MU) labeled substrates (Sigma−Aldrich Chemicals, Germany) as described in Pritsch et al. (2005), and with the following modifications of substrate concentrations and incubation times: 750 µM 4-MU-β-d-xylopyranoside for xylosidase (xyl, EC 3.2.1.37) and 120 min; 750 µM 4-MU-β-d d-glucuronide hydrate for glucuronidase (glr, EC 3.2.1.31) and 120 min; 300 µM 4-MU-β-d-cellobioside for cellobiohydrolase (cbh, EC 3.2.1.91) and 20 h; 750 µM 4-MU-N-acetyl-β-glucosaminide for N-acetyl-glucosaminidase (nag, EC 3.2.1.14) and 120 min; 600 µM 4-MU-β-d-glucopyranoside for β-glucosidase (gls, EC 3.2.1.3) and 120 min; and 1200 µM 4-MU-phosphate for phosphatase (pho, EC 3.1.3.2) and 30 min. The 100 µL of labeled substrate was mixed with 50 µL of soil suspension (three technical replicates per sample). The enzymatic reaction was stopped with 100 µL of 1 M Tris, pH > 10 (Pritsch et al. 2005). Possible autofluorescence or quenching of the fluorescence signal influenced by the soil was

Journal : Medium 11104 | Article No : 6564 | Pages : 22 | MS Code : 6564 | Dispatch : 23-2-2024

accounted for by using 50 µL of soil suspension of each sample and 100 µL containing 0–500 pmol MU as used for calibration(see below). Additionally, we included negative controls containing distilled water instead of soil suspension along with the respective substrate. Calibration curves were included in every measurement plate containing 50 µL each of sterile distilled water and 100 µL calibration solutions containing 0, 100, 200, 300, 400, 500 pmol MU, each. Prior to fluorescence measurements, the microplates were centrifuged for 5 min at 2500×g. Fluorescence measurements were performed on an Infinite M1000 Pro spectrofluorometer and accompanying i-control software (Tecan, Männedorf, Switzerland) at excitation/emission wavelengths of 365/450 nm. Released amounts of MU were calculated based on calibration curves (taking into account negative controls and quenching) and expressed as MU release in nmol per g soil dry weight and minutes (nmol g^{-1} min^{-1}).

To determine laccase activity (EC 1.10.3.2), soil suspensions were incubated with 500 µM 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) for 180 min. The plates were centrifuged to spin down particles for 5 min at 750×g and then the 250 µl without sediment were transferred into a new transparent plate. The intensity of green color was measured at 420 nm on an Infinite M1000 Pro spectrofluorometer and accompanying i-control software (Tecan, Männedorf, Switzerland). Laccase activities were expressed as the turnover of ABTS in nmol per g soil dry weight and min (nmol g^{-1} min^{-1}). Water instead of soil suspension was used as a negative control.

DNA extraction, PCR amplification, and sequencing

To assess the diversity and composition of soil fungal communities, DNA was extracted from 0.25 g of soil samples and five negative controls using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and using a Fastprep-24 (MP Biomedicals, Irvine, CA, USA) for bead beating (24 m s^{-1} 2×30 s). For targeting fungal ITS2 (internal transcribed spacer 2 rDNA), equimolar forward (ITS3 mix 1–5) and reverse primer (ITS4 mix 1–4) mixes were used according to Tedersoo et al. (2015). Primers carried overhangs for Illumina amplicon sequencing (Illumina protocol Part # 15044223; Illumina, San Diego, CA, USA) (Table S1). Reactions consisted of 1 µL DNA (5 ng), 0.5 µL 10 pmol ITS3tagmix, 0.5 µL 10 pmol ITS4tagmix, 10 µL NEBNext® High-Fidelity 2×PCR Master Mix (New England Biolabs, Frankfurt, Germany) and 8 µL H_2O. PCR conditions were 5 min at 95 °C, 28×[30 s at 95 °C, 30 s at 55 °C and 60 s at 72 °C] and 10 min at 72 °C. For each sample, three independent PCRs were run, and the quality of the products was assessed in 2% agarose gels. After pooling of the replicates, PCR products were cleaned using Agencourt AMPure XP (Beckman Coulter, Krefeld, Germany) at a 1:1 concentration according to the manufacturer's instructions. DNA concentrations were determined using an AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit (Biotium, Inc., Fremont, CA, USA).

Amplicons were indexed with using PCR with individual dual-index combinations of Nextera XT Index Kit v2 Sets A and B (Illumina) for each sample, and then cleaned, size-checked and quantified. The indexing PCRs contained 1 µl (c)DNA (5 ng), 2.5 µl Nextera i7 primer, 2.5 µl Nextera i5 primer, 12.5 µl NEBNext High-Fidelity 2X PCR MasterMix, and 6.5 µl ultra-pure H_2O. PCR conditions were 3 min at 95 °C, 8×[30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C] and 10 min at 72 °C. The final preparations and sequencing (Miseq v3 chemistry, 600 cycles flow cell, Illumina) followed the manufacturer's recommendations for ITS Metagenomic Sequencing Library Preparation (protocol Part # 15044223 Rev. B).

DNA sequence processing

Raw reads from Illumina MiSeq were processed with the automated pipeline PIPITS v2.7 (Gweon et al. 2015). Briefly, fungal sequences were prepared by joining read pairs and by quality filtering according to the pipeline's standard parameters. The ITS2 subregion was extracted using ITSx (Bengtsson-Palme et al. 2013). Short reads (< 100 bp) were removed, and sequences were assigned to operational taxonomic units (OTUs) with a 97% similarity threshold using VSEARCH (Rognes et al. 2016). Chimeric sequences were removed by comparison with the UNITE UCHIME database (v. 7.2, http://unite.ut.ee/repository.php). Taxonomic assignment to the level of species hypotheses (Nilsson et al. 2019) was performed using the RDP classifier (Wang et al. 2007) in combination with the UNITE fungal ITS database (v 8.2; Kõljalg et al. 2013; Abarenkov et al. 2020).

FungalTraits was used to identify different functional groups within the fungal communities (Põlme et al. 2020). It allows to assign fungal OTUs to trophic groups subdivided into specific guilds comprised of fungi that share similar lifestyle modes (e.g., ECM fungi, litter saprotrophs, soil saprotrophs, and root endophytes) and also to ECM "exploration type" (Agerer 2001) on genus level. Fungal OTUs assigned to SAPf were characterized by their major known capabilities and high saprotrophic potential according to their "primary lifestyle".

Soil parameter analysis

The water content of each soil sample was determined in triplicate (3 g each) after drying at 110 °C for 24 h and expressed as percent water content. Nitrate and ammonium were determined by the wet chemical method in triplicate as described in Nickel et al. (2017). Mineral nitrogen (N_{min}) was calculated as a sum of nitrate and ammonium.,

Statistical analysis

For analysis of the whole soil fungal community, OTUs with less than 10 total reads were considered potential contaminants and excluded from further analyses. Data were rarefied 1000 times using the 'rarefy' function (GNuniFrac, Chen et al. 2012) to a depth of 10,000 sequences per sample, and the results averaged. Analyses were conducted on the entire soil fungal community (ALLf) or on subsets of SAPf and ECMf.

Pair-wise correlations between soil parameters (soil water percentage and various nitrogen forms) and among various enzymes were assessed with Spearman correlation using the function '*cor.test*' (*stats*).

To check the normality of the data distribution, the Shapiro–Wilk test was used ('*shapiro.test*' in package *stats*). For normally distributed data, differences between mean values of α-diversity metrics were analyzed using an analysis of variance – ANOVA ('*aov*' in package *stats*) followed by Tukey's honest significance test ('*TukeyHSD*' in the *stats* package). Models included the pairs of experimental plots (TE-CO-) as random factor. When the assumptions of normality were not met, data were analyzed using the nonparametric Kruskal–Wallis test ('*kruskal.test*' in package *stats*), and then the differences were analyzed by Dunn's test for multiple comparisons ('*dunn.test*' in the *dunn.test* package).

The Shannon–Wiener diversity index and Simpson's index of diversity were calculated using the function '*diversity*' and species richness by using the function '*specnumber*' in *vegan* (version 2.5–7; Oksanen et al. 2021). Evenness was determined as Shannon Index/log(species count).

To investigate the dissimilarity between individual samples, for fungal communities, the Bray–Curtis dissimilarity and for enzyme activities, the Euclidean distance were calculated using the function '*vegdist*' in the *vegan* package. We used a permutational multivariate analysis of variance (PERMANOVA) using distance matrices with the function *'adonis2'* implemented in *vegan* to test the effects of the main factors (tree root zone, treatment and day relative to watering) and soil parameters on the fungal community composition. The nestedness of the experimental design was considered using the paired (control-rewatering) plots as random factor and for the *'strata'* option. To uncover significant differences between factors (treatment, root zone, and day relative to watering), we used multilevel pairwise comparisons of permutational multivariate analysis of variance (pairwise PERMANOVA), function '*pairwise.adonis'* (Martinez Arbizu 2020) with Bonferroni p value correction. Fungal community composition and enzyme profiles were visualized using principal coordinate analysis (PCoA) ordinations. The package *phyloseq* (McMurdie and Holmes 2013) was used create charts on relative abundances.

To estimate the dynamics of fungal communities in the rewatering treatment relative to the control treatment (averaged by "day relative to watering"), the standard error of the difference between means ($\sigma_{M_1-M_2}$) was calculated according to Foster et al. (2018) as follows:

$$\sigma_{M_1-M_2} = \sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}$$

where σ_1^2 and σ_2^2 describe the respective variances of control and rewatering samples and replicate number (n_1 and n_2).

Mantel tests were used to test correlations between variations in enzyme activities and variations in fungal community composition in the R package *ecodist*

Journal : Medium 11104 | Article No : 6564 | Pages : 22 | MS Code : 6564 | Dispatch : 23-2-2024

(Goslee and Urban 2007) given by the Bray–Curtis index as a dissimilarity metric. Pearson correlations between the relative abundance of genera in samples and enzyme activities were established using the R package *Hmisc* (Harrell and Harrell 2019).

For each genus, a set of specific enzyme activities $E_{S,X}$ were calculated as abundance weighted averages with the formula described by Bödeker et al. (2014) as follows:

$$E_{s,x} = \frac{\sum_{i=1}^{n} E_{xi} Pi}{\sum_{i=1}^{n} Pi}$$

where E_{xi} is the activity of enzyme X in sample i, Pi is the relative amplicon abundance of genera in sample i and n is the total number of samples.

All statistical analyses were performed using R (version 4.1.2, RCore Team 2021) and RStudio Desktop (version 2021.09.2–382, RStudio Inc.). P values < 0.05 were considered statistically significant.

Results

Abiotic soil parameters

Both, total soil nitrogen content and N_{min} were significantly affected by all three factors, treatment, root zone and day relative to watering (Fig. S1, Table S2). Treatment and day relative to watering significantly influenced ammonium concentrations, while nitrate concentrations were influenced by the root zone (Table S2). All inorganic nitrogen forms were highly correlated (Fig. S2). The soil water content reached the same level in the rewatering and control samples at different time points according to root zone: at 7 d in the spruce zone, at 42 d in the beech zone, and with an unclear pattern in the Mix zone (higher in rewatered than control at 42d, 84 d) (Fig. S1).

Sequencing output

A total of 2,824,111 fungal sequences were obtained and assigned to 3,966 OTUs. The average sequencing depth of the samples was 23,466 reads. Eleven samples of the real samples had readings below 10,000 and were excluded from further analysis. The most abundant phyla were Ascomycota (34% of fungal OTUs, 20% of fungal sequences), Basidiomycota (21%, 54%), Mortierellomycota (3%, 16%), Mucoromycota (2%, 2%), Rozellomycota (2%, 2%), and Chytridiomycota (1%, < 1%). Considering the primary fungal lifestyle, 896 OTUs (23% of fungal OTUs, 34% of sequences) were assigned to SAPf, followed by ECMf (6%, 39%), pathogenic fungi (1%, < 1%), others (3%, < 1%), and fungi of unknown lifestyle (67%, 26%). In the SAPf, fungi that also had root endophyte ability represented 2.8% of all sequences, and 0.2% of sequences could be assigned to ectomycorrhizal fungi (Fig. 1).

Diversity of soil fungi in response to rewatering in different root zones

Treatment had no effect on any of the α-diversity indices of ALLf (Table 1). However, Pielou's evenness was higher for SAPf in rewatered than in control (Table S3). Greater Shannon diversity and species richness were observed for ECMf under control conditions than under rewatered conditions ($P < 0.05$) (Table 1, Table S3).

Tree root zone significantly affected all α-diversity indices for ALLf (Shannon – $P < 0.001$, ANOVA; Simpson – $P < 0.001$, Kruskal–Wallis; Pielou evenness—$P < 0.001$, ANOVA) (Table 1). ALLf communities from spruce zone of rewatering and controls, were characterized by the highest Shannon index (mean H = 4.35) in contrast to the beech root zone with the lowest (mean H = 3.84) and intermediate values for the Mix root zone (mean H = 4.02) (Table 1, Table S3). Similar results were obtained for the other α-diversity indices (Simpson index, Pielou Eveness) for ALLf (Table S3, S4). Separated by trophic mode, SAPf diversity indices followed the same pattern as those of ALLf; however, for ECMf, Shannon, Simpson, and Pielou evenness were not influenced by root zone (Table 1; Tables S3, S4).

'Day relative to watering' had no significant effect on the diversity of ALLf, SAPf, or ECMf (Table 1).

Composition of soil fungal communities following rewatering

Tree root zone and treatment significantly affected the community composition of ALLf, SAPf and ECMf (Table 2, S5). Principal Coordinate Analysis indicated that groupings according to these factors were evident for ALLf (Fig. 2a). In contrast to

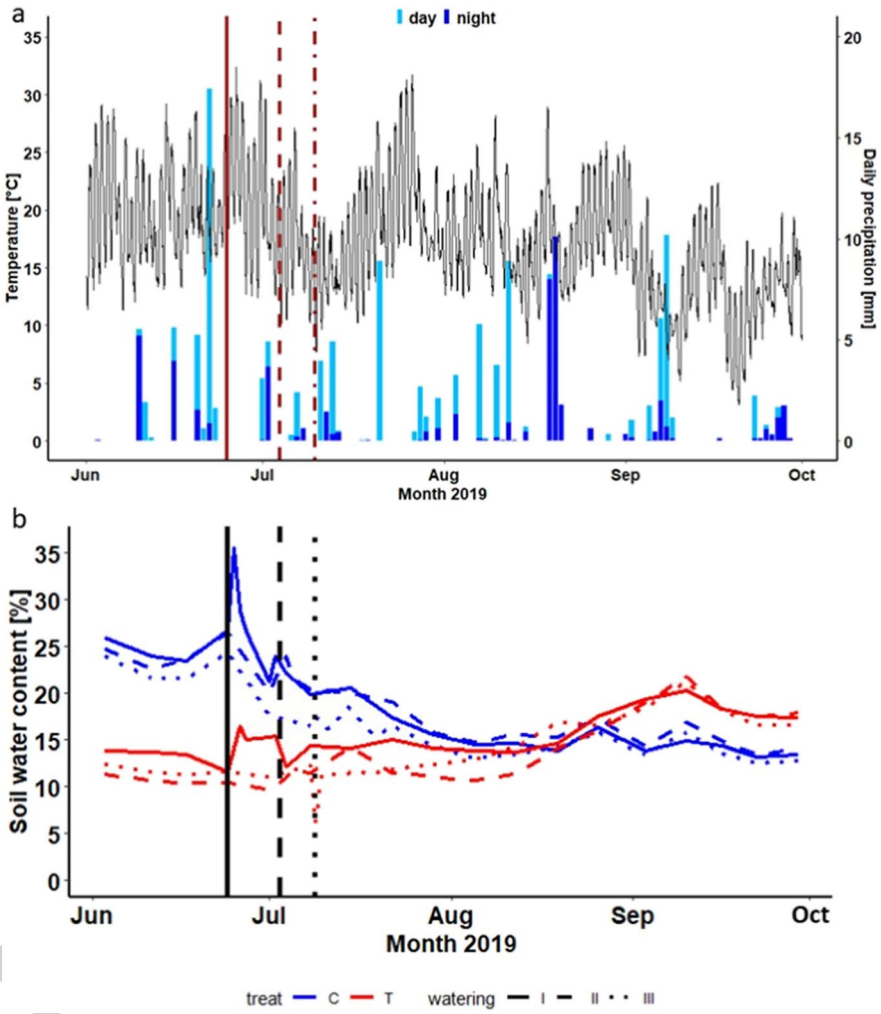

Fig. 1 Dynamics of changes in temperature and precipitation during day (light blue) and night (dark blue) at the experimental plots during the rewatering experiments (**a**). Volumetric soil water content (**b**) in 0 – 7 cm of control (blue) and rewatered plots (red) whereas the line types indicate the plots of different watering campaigns (solid = first, broke = second and dotted = third watering campaign) within the sampling period. The vertical lines on both figures specify the days of the watering events

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Table 1 Effects of treatment, root zone and day relative to watering on different α-diversity indices calculated for all soil fungi (ALLf), saprotrophic (SAPf), and ectomycorrhizal fungi (ECMf)

Diversity indices	Fungal group	Treatment (df = 1)	Root zone (df = 2)	Day relative to rewatering (df = 4)
Shannon index	ALLf	F = 2.48, $P = 0.119$	**F = 39.45, $P < 0.001$**	F = 0.12, $P = 0.730$
	SAPf	$\chi^2 = 2.40$, $P = 0.122$	**$\chi^2 = 33.92$, $P < 0.001$**	$\chi^2 = 4.96$, $P = 0.292$
	ECMf	**F = 7.45, $P < 0.01$**	F = 0.06, $P = 0.939$	F = 1.55, $P = 0.215$
Simpson index	ALLf	$\chi^2 = 0.16$, $P = 0.687$	**$\chi^2 = 31.80$, $P < 0.001$**	$\chi^2 = 2.58$, $P = 0.629$
	SAPf	$\chi^2 = 3.40$, $P = 0.065$	**$\chi^2 = 28.22$, $P < 0.001$**	$\chi^2 = 5.38$, $P = 0.251$
	ECMf	$\chi^2 = 1.64$, $P = 0.199$	$\chi^2 = 1.43$, $P = 0.488$	$\chi^2 = 3.07$, $P = 0.546$
Pielou Eveness	ALLf	F = 0.10, $P = 0.749$	**F = 29.94, $P < 0.001$**	F = 0.06, $P = 0.802$
	SAPf	**$\chi^2 = 6.49$, $P < 0.05$**	**$\chi^2 = 20.33$, $P < 0.001$**	$\chi^2 = 1.95$, $P = 0.745$
	ECMf	$\chi^2 = 0.11$, $P = 0.740$	$\chi^2 = 3.55$, $P = 0.169$	$\chi^2 = 0.81$, $P = 0.937$

Comparison of diversity indices for the factors treatment (control vs. rewatered drought treated), different tree root zone (spruce-monospecific, beech-monospecific, mixture zone between beech and spruce), and day relative to start of rewatering (day -7, 7, 18, 42, and 84): when data passed the Shapiro test for normal distribution, ANOVA was applied, if not the Kruskal–Wallis test was used. Significant differences are highlighted in bold ($P < 0.05$)

Table 2 PERMANOVA on the composition of all fungi (ALLf), soil saprotrophic fungi (SAPf), and ectomycorrhizal fungi (ECMf) based on Bray-Curtis dissimilarities following the overall experimental design AQ11

Factor	df	ALLf		SAPf		ECMf	
		R^2	P	R^2	P	R^2	P
Treatment (T)	1	**0.065**	**< 0.0001**	**0.085**	**< 0.0001**	**0.050**	**< 0.0001**
Root zone (RZ)	2	**0.18**	**< 0.0001**	**0.218**	**< 0.0001**	**0.164**	**< 0.0001**
Soil Water Content (SWC)	1	**0.01**	**0.0465**	0.009	0.1286	**0.014**	**0.0178**
N_{min} (N)	1	**0.01**	**0.0184**	**0.017**	**0.0134**	0.012	0.1266
T x RZ	2	**0.04**	**0.0003**	**0.030**	**0.0020**	**0.044**	**0.0002**
RZ x N	2	**0.02**	**0.0378**	**0.026**	**0.0094**	0.020	0.1442

Effect of tree species (root zone, RZ), control and rewatering (treatment, T), soil water content (SWC) and soil N_{nim} (N) and their interactions, excluding insignificant terms. Full model with all factors in Table S5

ECMf, the clustering of the SAPf among the investigated factors was very pronounced (Fig. 2b, c). Day relative to watering had no significant effect (Table 2, S5). The community composition in the different (spruce, beech and mix between them) root zones responded differently to the treatment for ALLf, SAPf and ECMf, as indicated by the significant root zone × treatment interaction (Table 2, Table S6). A pairwise comparison between beech, spruce and Mix root zones showed that the community composition differed between beech and spruce soils and that the composition of communities from the Mix root zone was more similar to the beech root zone than to spruce root zone (Table S7). Both, soil water content at the time of sampling ($P = 0.0465$, PERMANOVA) and N_{min} ($P = 0.0184$, PERMANOVA) had significant effects on the ALLf community, whereas N_{min} only had a significant effect on the SAPf community and soil water content only had a significant effect on ECMf community composition (Table 2, Table S7).

In the beech root zone, higher relative abundances of SAPf in rewatered samples than in control were observed directly after rewatering, which declined within two weeks, while the abundance of ECMf increased at the same time (Fig. 3a, b). In the spruce root zone, the relative abundance of ECMf in the rewatered samples was lower than that of control most of the time but returned to the level of control at the end of the experiment, in contrast to the abundance of SAPf (Fig. 3a, b). From 18 d after rewatering and onwards, the abundance of ECMf in the Mix root zone increased and remained above control levels until the end of the sampling period (Fig. 3a, b).

Among SAPf, the subgroup of soil saprotrophs was dominant in all samples (Fig. 3c). However, in the beech root zone, the relative amounts of litter saprotrophs in rewatered samples decreased from 18% 7 d before rewatering to 10% 7 d after rewatering in favor of soil saprotrophs (Fig. 3b, d). Apart from this fluctuation, the difference was very small (c. 6%) between sampling dates relative to control (Fig. 3b, d).

The relative abundances of exploration types of the ECMf communities changed differently in rewatered samples relative to control of the different root zones after rewatering (Fig. S3).

In the beech root zone of the rewatering, medium-distance fringe types were absent before rewatering (Fig. S3a) but were seen as a stable community component (c. 6% lower than in control) afterwards (Fig. S3b). This mostly happened at the expense of the contact exploration types, which dominated at all times (Fig. S3a). Long distance exploration types were a minor component of the beech root zone in the rewatered community before rewatering and were even less abundant after rewatering (Fig. S3a). While the distribution of exploration types in the beech zone differed between control and rewatered samples before rewatering, the two became similar at the end of the observation period (Fig. S3b).

In the Mix root zone, contact types also dominated at all times, with minor fluctuation. Exploration types from the Mix root zone of rewatered samples resembled those of control, albeit with a higher share of contact types and a lower share of short distance types (Fig S3b).

In the spruce root zone, medium-distance fringe-type taxa were absent in rewatered samples, while

Journal : Medium 11104 | Article No : 6564 | Pages : 22 | MS Code : 6564 | Dispatch : 23-2-2024

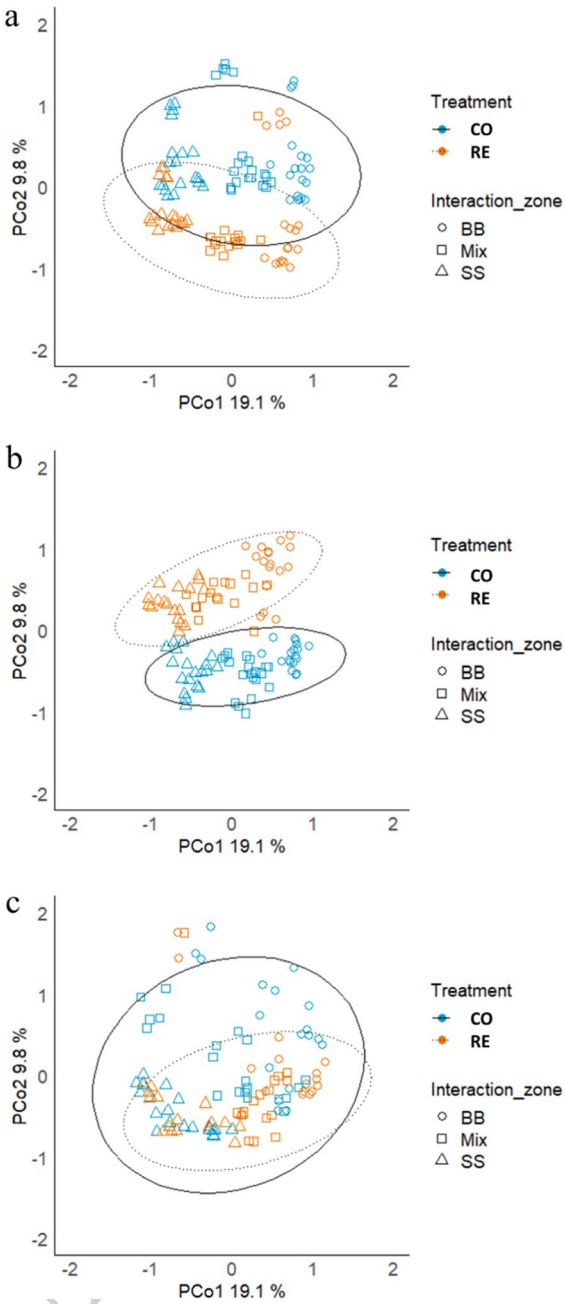

Fig. 2 Principal Coordinate Analysis based on fungal community dissimilarity (Bray-Curtis) for ALLf (**a**), SAPf (**b**) and ECMf (**c**). Each point represents the soil fungal community of one sample. Ellipses: 95% confidence interval for samples from control (CO, solid line, blue points) and rewatered (RE, dotted line, red points). Shapes: tree root zones of beech-beech (round BB), spruce-spruce (triangle SS) and beech-spruce mixture (square Mix)

they made up a small fraction (3%) in control. Similarly, short-distance-type taxa were mainly lower in rewatered samples than in control. Long-distance exploration types were more abundant in rewatered samples (approximately 3% higher than in control) than in control before rewatering and even increased to 4–8% after rewatering in rewatered relative to the control (Fig. S3b) compared to c. 4% in control (Fig. S3a).

Among the SAPf, no changes in abundance were observed between rewatering and control soil in the spruce root zone (Fig. S4). Only 3 genera differed in relative abundances between control and rewatered samples in the beech root zone, with relatively higher abundances in rewatered samples for *Geomyces* and *Solizoccozyma* ($P < 0.05$, Kruskal−Wallis) and the opposite for *Mortierella* (control > rewatered, $P < 0.05$, Kruskal−Wallis). In the Mix root zone, the genera *Absidia*, *Geomyces*, *Oidodendron*, *Rhodocollybia*, *Solizoccozyma*, *Trechiospora* and *Umbelopsis* were more abundant in rewatered samples than in control ($P < 0.05$, Kruskal−Wallis). Although the samples were isolated from soil, some of the taxa classified as saprotrophic also show a different lifestyle. For example, *Archaeorhizomyces*, *Mortierella*, and *Umbelopsis* may also be root-associated fungi, and *Oidiodendron* may function as a root endophyte (Fig. S4).

Comparing ECMf genera, *Amanita* was more abundant in rewatered samples than in control in all zones ($P < 0.05$), *Thelephora* was more abundant in the Mix and spruce root zones, and *Xerocomellus* was more abundant only in the spruce zone (Fig. S4). Some ECMf genera were more abundant in control than in rewatered samples: *Cortinarius* in the spruce and Mix root zones and *Lactarius*, *Piloderma,* and *Tylospora* in the spruce zone.

Soil enzyme activities

Treatment and tree root zone both had a significant effect on all measured soil enzyme activities, but the day after watering did not (Table 3).

Five out of seven enzymes significantly differed between rewatering and control (Tables 3 and 4), with nag and lac activities being higher in control than in rewatered plots. The two enzymes gls and glr had higher activity in the rewatered plots than in the control plots. The greatest difference between

Journal : Medium 11104 | Article No : 6564 | Pages : 22 | MS Code : 6564 | Dispatch : 23-2-2024

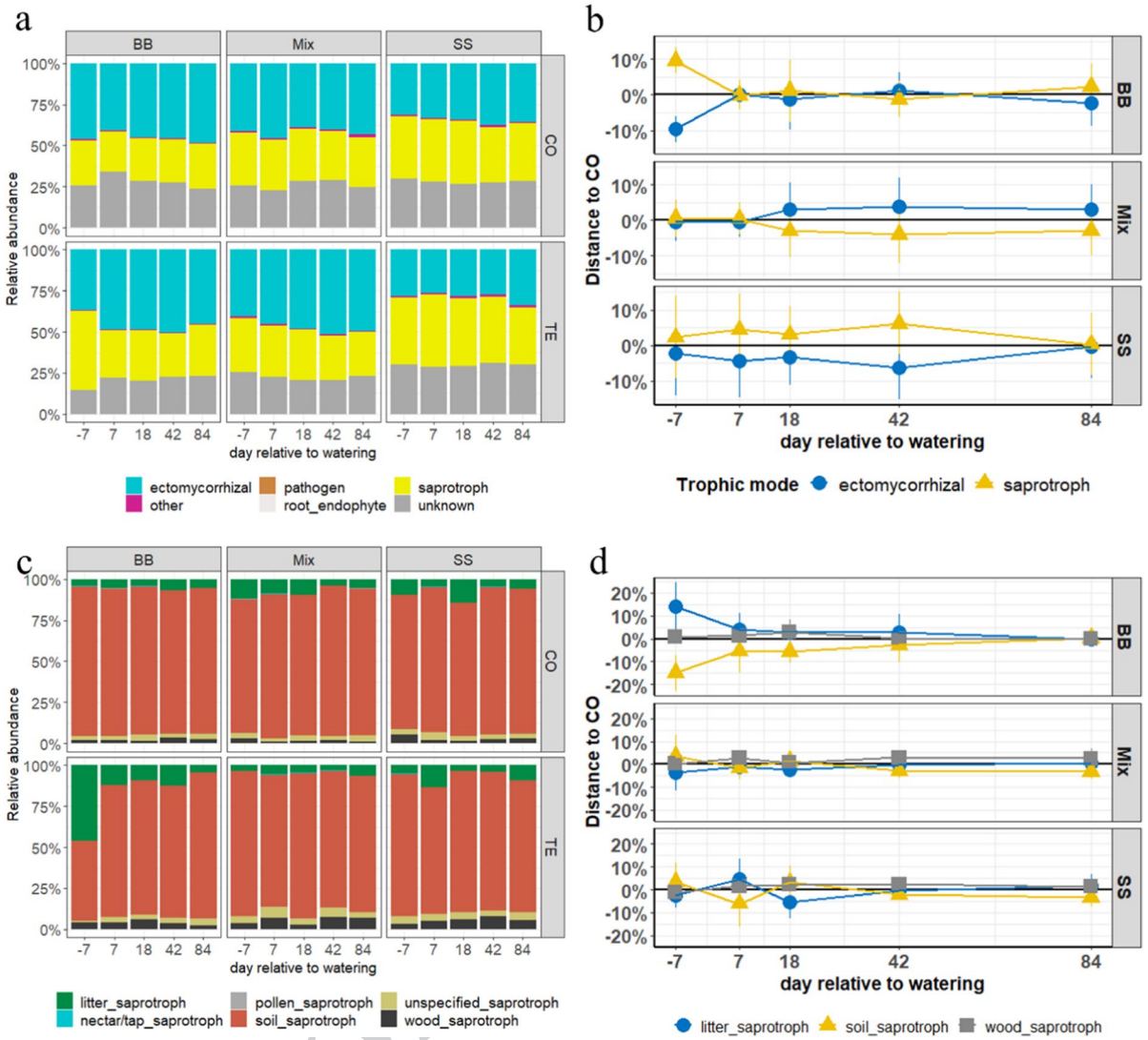

Fig. 3 Time course of relative abundances of different soil fungal groups in control (CO), and rewatering plots (TE) relative to watering (day -7, 7, 18, 42, and 84) and in three root zones (SS – spruce monospecific, BB- beech monospecific, Mix-mixture zone of both). Left panels (**a**, **c**) relative shares of a) all fungal groups; c) fungal saprotrophic groups; right panels (**b**, **d**) changes in TE (colored lines) relative to CO (black line) based on average values ± standard errors of the difference between means of b) ectomycorrhizal and saprotroph fungi; d) litter, soil, wood saprotrophs. "other" sums up the following fungal groups: algal parasite, animal parasite, mycoparasite, root endophyte, epiphyte, foliar endophyte, lichenized, arbuscular mycorrhizal

rewatered and control (higher in rewatered plots) was observed in soil samples from the beech root zone for enzyme activities related to cell wall degradation (cbh, glr, gls, xyl) (Fig. S5). For these enzymes, higher activities were also observed in the Mix root zone. In the spruce root zone, only cbh and gls had higher activities in the rewatered plots than in the control plots.

The tree root zone had the greatest influence of the tested factors on most soil enzyme activities in control and rewatered plots, except for pho (Table 4). The activities of enzymes related to cell wall degradation were highest in the spruce root zone and lowest in the beech root zone, with intermediate activity in the Mix root zone (Table 5). Nag activity was significantly higher in samples from the spruce root zone,

Table 3 Soil enzyme activities (pho—phosphatase; nag—N-acetyl-glucosaminidase; gls—β-glucosidase; cbh—cellobiohydrolase; glr—glucuronidase; xyl—xylosidase; lac – laccase) as affected by. treatments (CO and RE), three different root zones (spruce – spruce, beech –beech, and mixture zone between the two species), and time of watering (-7, 7, 18, 42, and 84 after starting rewatering): [a] – for normally distributed data ANOVA was applied; [b] – for non-normal distributed data Kruskal–Wallis test

enzyme	Treatment (df = 1)	Root zone (df = 2)	Day relative to rewatering (df = 4)
pho	$\chi^2 = 2.81$, $P = 0.0935$	$\chi^2 = 2.18$, $P = 0.3367$	$\chi^2 = 5.17$, 0.2702
nag	**$\chi^2 = 4.71$, $P = 0.0301$**	**$\chi^2 = 10.21$, 0.0061**	$\chi^2 = 4.63$, $P = 0.3278$
gls	**$F = 8.13$, $P = 0.0053$**	**$F = 66.43$, $P < 0.0001$**	$F = 0.05$, $P = 0.8164$
cbh	**$F = 7.10$, $P = 0.0089$**	**$F = 58.52$, $P < 0.0001$**	$F = 0.16$, $P = 0.6920$
glr	**$F = 5.19$, $P = 0.0248$**	**$F = 48.06$, $P < 0.0001$**	$F = 0.05$, $P = 0.8263$
xyl	$\chi^2 = 0.65$, $P = 0.4219$	**$\chi^2 = 64.84$, $P < 0.0001$**	$\chi^2 = 4.63$, $P = 0.3278$
lac	**$F = 17.03$, $P < 0.0001$**	**$F = 4.46$, $P = 0.0139$**	$F = 0.06$, $P = 0.8017$

Significant differences in bold ($p < 0.05$). To achieve normal distribution the data were log transformed

Table 4 Soil enzymes activities according to treatment (CO – control, RE – previously drought treated plots) and root zone (RZ: BB – beech, Mix – mix zone, SS – spruce), values are given as mean with SE in brackets, and are expressed as release of methylumbelliferone (MU) release (pM/mg dry soil/min) for pho—phosphatase, nag—N-acetyl-glucosaminidase, gls—β-glucosidase, cbh—cellobiohydrolase, glr—glucuronidase, xyl—xylosidase; and as ABTS turnover (nM/g dry soil/min) for lac—laccase

	RZ	pho	nag	gls	cbh	glr	xyl	lac
CO	BB	32.32 (2.29)	5.38 (0.53)	8.50 (1.28)	1.02 (0.18)	1.24 (0.40)	4.16 (0.58)	267.33 (32.33)
	Mix	33.70 (3.66)	6.13 (1.15)	15.35 (1.98)	1.69 (0.26)	1.77 (0.24)	9.43 (1.23)	222.05 (36.13)
	SS	39.55 (3.12)	12.90 (3.86)	36.19 (3.34)	4.80 (0.71)	3.30 (0.19)	18.19 (0.95)	213.74 (18.56)
RE	BB	33.98 (3.62)	7.24 (0.81)	12.81 (1.18)	1.47 (0.14)	1.37 (0.14)	6.28 (0.70)	180.35 (24.97)
	Mix	27.98 (2.37)	7.40 (0.81)	21.31 (2.38)	2.48 (0.35)	2.46 (0.31)	11.14 (1.13)	115.63 (22.61)
	SS	32.55 (4.44)	9.05 (1.28)	33.03 (2.52)	4.50 (0.50)	3.48 (0.38)	15.99 (1.43)	161.33 (14.68)

Table 5 Correlation between soil enzyme activities and SAPf and ECMf diversity and composition (PCoA1, PCoA2)

		SAPf			ECMf		
		Shannon diversity	PCoA1	PCoA2	Shannon diversity	PCoA1	PCoA2
Nutrient acquring	pho						
	nag	0.31	-0.39			-0.11	
hydrolytic	cbh	0.56	-0.78			-0.42	-0.46
	xyl	0.49	-0.81			-0.37	-0.58
	gls	0.54	-0.81			-0.39	-0.53
	glr	0.51	-0.77			-0.36	-0.52
oxidative	lac		0.19	-0.41		-0.38	0.32

Only significant relationships ($P < 0.05$) are marked with Spearman correlation coefficients. pho—phosphatase; nag—N-acetyl-glucosaminidase; gls—β-glucosidase; cbh—cellobiohydrolase; glr—glucuronidase; xyl—xylosidase; lac – laccase

and there were no differences between beech and Mix root zones for this enzyme. In contrast, lac had the highest activity in the beech zone, with no differences between the spruce and Mix root zones (Table 5, Fig. S5).

Soil enzyme activity profiles partly separated soil samples in principal coordinate analysis (Fig. 4). Enzyme activity profiles correlated with fungal community composition: ALLf (rho = 0.47, $P < 0.001$, Mantel test), SAPf (rho = 0.42, $P < 0.001$, Mantel test), and ECMf (rho = 0.34, $P < 0.001$, Mantel test). Analyzing control and rewatered samples separately using Mantel tests, soil enzyme activity profiles correlated with higher values for SAPf (rho = 0.50, $P < 0.001$, Mantel test) than EMCf (rho = 0.29, $P < 0.001$, Mantel test) in rewatered plots, while enzyme profiles of control similarly correlated with SAPf (rho = 0.43, $P < 0.001$, Mantel test) and ECMf (rho = 0.41, $P < 0.001$, Mantel test). Moreover, significantly more SAPf taxa than ECMf taxa correlated with soil enzyme activities (Table 6). The relative abundance of SAPf taxa was positively correlated with most enzymes, particularly with C-compound degrading enzymes (cbh, xyl, gls, glr, lac), and significantly less correlated with nag and pho. All 10 of the most abundant genera of SAPf were positively correlated with different soil enzyme activities (Table 6). Additionally, some ECMf genera correlated positively with soil enzyme activities (*Elaphomyces, Pseudotomentella, Tylopilus, and Tylospora*), while most were negatively associated (*Cenococcum, Clavulina, Lactarius, Melanogaster, Piloderma, Russula, Thelephora*).

Discussion

Response of SAPf vs. ECMf communities to rewatering

Spruce ECMf communities did not rapidly change, resembling the dynamics of ECMf on regenerating spruce fine roots after rewatering (Danzberger et al. 2023). In our study, we observed an increase in long distance exploration type in spruce soil during rewatering. Although these exploration types, together with medium distance mat, and medium distance fringe, are characterized by increased nitrogen uptake, unlike the others (Hobbie and Agerer 2010), this may not be the result of chitinase production,

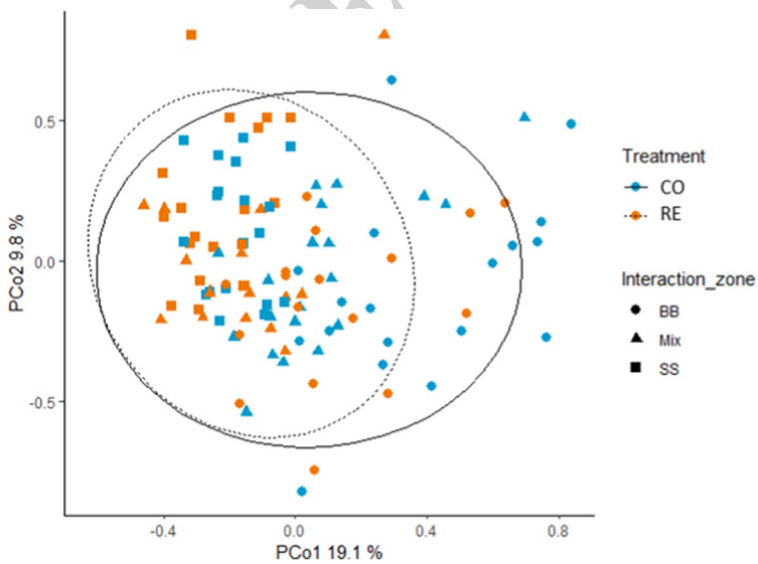

Fig. 4 Ordination plot of the first (x-axis) and the second dimensions (y-axis) of principal coordinate (PCoA) scores for soil enzyme activity profiles with each dot representing seven enzymes (phosphatase, N-acetyl-glucosaminidase, β-glucosidase, cellobiohydrolase, glucuronidase, xylosidase, laccase). Ellipses show 95% confidence interval for samples from control (CO, solid line, blue points) and rewatering plots (RE, dotted line, red points). Symbols represent soil samples from three root zones: beech-beech (round BB), spruce-spruce (square SS) and beech-spruce mixture (triangle Mix)

Journal : Medium 11104 | Article No : 6564 | Pages : 22 | MS Code : 6564 | Dispatch : 23-2-2024

Table 6 Correlation between relative abundance of a genus and soil enzyme activity

Genus	Primary lifestyle	Secondary lifestyle	Endophytic interaction capability	pho	nag	gls	cbh	glr	xyl	lac
Cenococcum	ectomycorrhizal	-						-0.18	-0.21	0.26
Clavulina	ectomycorrhizal	-				-0.18	-0.19	-0.20		
Elaphomyces	ectomycorrhizal	-			0.24			0.26	0.29	0.33
Lactarius	ectomycorrhizal	-				-0.23	-0.24		-0.26	
Leotia	ectomycorrhizal	-								0.19
Melanogaster	ectomycorrhizal	-						-0.18	-0.21	
Piloderma	ectomycorrhizal	-			-0.18	-0.27	-0.28	-0.28	-0.26	-0.31
Pseudotomentella	ectomycorrhizal	-				0.31			0.24	
Russula	ectomycorrhizal	-				-0.33	-0.19	-0.28		0.34
Thelephora	ectomycorrhizal	-								-0.29
Tomentella	ectomycorrhizal	-							0.26	
Tylopilus	ectomycorrhizal	-				0.20			0.25	
Tylospora	ectomycorrhizal	-				0.25	0.24	0.26	0.31	
Amaurodon	litter saprotroh	-			0.29	0.23				
Byssonectria	litter saprotroph	-				0.26	0.18	0.21	0.28	
Cadophora	litter saprotroph	-	ectomycorrhizal				0.20		0.18	
Chaetosphaeria	litter saprotroph	wood saprotroph	foliar endophyte			-0.27	-0.24	-0.25	-0.24	
Entoloma	litter saprotroph	-	ectomycorrhizal			0.25		0.28	0.31	
Hyaloscypha	litter saprotroph	wood saptroph	ectomycorrhizal							0.20
Leptodontidium	litter saprotroph	ericoid mycorrhizal	root endophyte							-0.26
Maasoglossum	litter saprotroph	-							0.25	
Pseudopenidiella	litter saprotroph	-		0.27		0.24	0.28			
Rhodocollybia	litter saprotroph	-		0.26	0.33		0.20			
Ripartites	litter saprotroph	-		0.20					0.28	
Absidia	soil saprotroph	-					0.32	0.27		-0.18
Archaeorhizomyces	soil saprotroph	root associated	root associated			0.26	0.20	0.19	0.26	
Cladophialophora	soil saprotroph	-	root endophyte		0.27					
Geomyces	soil saprotroph	-							0.21	-0.21
Glarea	soil saprotroph	-		0.18				0.18	0.24	
Goffeauzyma	soil saprotroph	plant associated							0.23	
Mortierella	soil saprotroph	root associated	root associated		-0.26		-0.27			
Mucor	soil saprotroph	-						0.19	0.21	
Oidiodendron	soil saprotroph	root endophyte	root endophyte					0.29		-0.21
Phallus	soil saprotroph	-				0.28		0.26		
Phialocephala	soil saprotroph	root endophyte	root endophyte					0.34		
Ramicandelaber	soil saprotroph	-					-0.30	-0.32		
Solicoccozyma	soil saprotroph	epiphyte					0.24			
Umbelopsis	soil saprotroph	root associated	root associated	-0.25	-0.24					
Basidiobolus	unspecified saprotroph	-		0.34		0.20	0.26		0.30	
Brachysporium	unspecified saprotroph	-							-0.19	0.25
Chalara	unspecified saprotroph	wood pathogen	foliar endophyte	0.22		0.28	0.31	0.31	0.28	
Lasiosphaeris	unspecified saprotroph	-								0.30
Penicillium	unspecified saprotroph	-	foliar endophyte			0.29	0.26	0.33	0.34	-0.32
Rhodotorula	unspecified saprotroph	foliar endophyte	foliar endophyte						0.18	
Sagenomella	unspecified saprotroph	-								-0.19
Slooffia	unspecified saprotroph	-							0.19	
Talaromyces	unspecified saprotroph	-				0.28	0.30	0.29	0.30	-0.21
Tritirachium	unspecified saprotroph	animal pathogen			0.34	0.29				
Acericola	wood saprotroph	-								0.22
Arachnopeziza	wood saprotroph	-		-0.19						
Ascocorticium	wood saprotroph	-				0.19			0.24	
Ciliciopodium	wood saprotroph	litter saprotroph		0.20	0.21	0.24	0.32	0.21		0.19
Connersia	wood saprotroph	-								0.21
Cristinia	wood saprotroph	-							0.18	
Diplococcium	wood saprotroph	litter saprotroph								0.18
Hyphoderma	wood saprotroph	-		0.21						
Hypholoma	wood saprotroph	-		0.20						
Lophiostoma	wood saprotroph	litter saprotroph							0.19	
Neobulgaria	wood saprotroph	-							-0.19	
Phragmocephala	wood saprotroph	-				-0.20		-0.18	-0.22	
Rigidoporus	wood saprotroph	-								
Scytalidium	wood saprotroph	-				-0.18				-0.18
Trechispora	wood saprotroph	-				0.33	0.30	0.30		-0.19
Spizellomyces	pollen saprotroph	-			0.25					
Terramyces	pollen saprotroph	-				-0.19	-0.20	-0.20		
Exophiala	animal pathogen	litter saprotroph	root endophyte			0.25		0.19		
Sugiyamaella	animal endosymbiont	necter/tap saprotroph				0.30		0.27	0.28	
Cutaneotrichosporon	animal parasite	animal decomposer						-0.20	-0.18	
Haptocillium	animal parasite	animal decomposer							0.22	0.21

Table 6 (continued)

Monacrosporium	animal parasite	wood saprotroph			-0.32	-0.30	-0.27	-0.26	
Ochroconis	animal parasite	plant pathogen		0.18	0.20	0.31	0.23	0.21	
Phialemonium	animal parasite	-					-0.18	-0.19	
Pochonia	animal parasite	animal decomposer						0.20	
Polycephalomyces	animal parasite	-			0.22				
Mycosymbioces	mycoparasite	fungal decomposer							-0.21
Sepedonium	mycoparasite	-					0.23		
Tremella	mycoparasite	-			-0.18			-0.22	0.26
Porosphaerella	foliar endophyte	litter saprotroph	foliar endophyte						0.19
Pezizella	root endophyte	-	root endophyte	0.25					
Pezoloma	root endophyte	soil saprotroph	root endophyte		0.21				
Colpoma	plant pathogen	litter saprotroph						0.21	
Ilyonectria	plant pathogen	-			0.19		0.19	0.28	-0.19

Only genera accounting for more than 1% of all reads in two or more samples were included. The numbers in the table show the Pearson correlation coefficient

but better competition and protection of the occupied area (Mucha 2011). Fungi that make up the contact, short and medium smooth exploration type have a smaller biomass of extramatrical mycelium (Agerer 2001). According to Tedersoo and Smith (2013) fungi forming exploration types with smaller extramatrical mycelium regenerate faster in response to environmental disturbances, and in our study they were more abundant in soil of beech and Mix root zone in rewatered plots than in control. High turnover of fine roots (dieback and regrowth) during a previous severe natural drought (Nikolova et al. 2020) provided a dynamic habitat for ECMf with continuously recovering beech roots to be colonized in the beech and Mix root zones. Accordingly, ECMf communities had a high potential to recover under rewatering conditions (Danzberger et al. 2023). As a consequence of a substantial accumulation of litter over the 5 drought years (personal observation) in the beech root zones of rewatered plots, rewatering resulted in a rapid turnover of litter decomposer communities to more soil saprotrophic communities within two weeks. This is in agreement with the fast decay of high-quality beech litter (Berger and Berger 2014) and the natural turnover of decomposer communities with changing substrates in litter after drought (Asplund et al. 2018). SAPf in our study also followed different dynamics in beech soils with a decrease in litter decomposers one week after rewatering, which was not the case in spruce soils. This may be explained by the initial hydrophobicity of spruce litter and its overall higher recalcitrance due to its high content of phenolic compounds (Thai et al. 2023). Aligning with our results, SAPf richness and diversity in soils were found to be higher under conifers than under beech trees (Cornelissen et al. 2001; Kubartová et al. 2009), which the authors attributed to a more recalcitrant litter quality in conifers requiring a more diverse enzyme profile for decay. The faster dynamics of changes in saprotrophic fungal communities combined with their greater production of enzymes may result in a faster availability of released nutrients necessary for root regeneration in soil with beech litter.

In our study, N_{min} significantly increased in accordance with other drought experiments that found reduced mineralization and nitrification under drought (Deng et al. 2021). In addition, the variation in N_{min} with different root zone confirms an influence of litter (amount and/or quality) on mineral nitrogen release (Martínez-García et al. 2021). Among the measured soil abiotic factors, soil moisture content was mainly associated with ECMf, in contrast to H1. $N_{min,}$ on the contrary, was associated with SAPf. This seemingly contradicts results from a Mediterranean forest where soil SAPf was more affected by drought than ECMf (Castaño et al. 2018). However, a recent study showed that ECMf biomass is mainly driven by soil temperature, moisture and pH, while SAPf biomass is associated with soil organic C and the C:N ratio and forest attributes (tree basal area and proportion of harvested tree biomass) (Awad et al. 2019). Moreover, our finding of a positive correlation between SAPf abundance and N_{min} is consistent with the results of another study showing that the accumulation of nitrogen along with organic matter can drive the abundance of saprotrophic fungi (Morrison et al. 2016).

Some fungal species may have more than one nutritional mode reflected in their lifestyle, as they may occupy more than one ecological niche (Lofgren et al. 2018; Martino et al. 2018). One of the dominant genera in our study, *Archaeorhizomyces*, is

widespread in diverse ecosystems worldwide (Alburae et al. 2020). However, they do not produce recognizable mycorrhizal structures and show saprotrophic potential in the decomposition of organic compounds (Rosling et al. 2011), and information on this class of fungi is still very limited and the mode of nutrition remains uncertain. *Oidiodendron* is another taxon with an ambiguous lifestyle. The nutritional mode of *Oidiodendron* is unclear and could be either saprotrophic or symbiotrophic, as it is isolated from decaying plant material (Calduch et al. 2004) and in ericoid species, improves nitrogen uptake and plant growth (Wei et al. 2016). Considering the proximity of niches such as roots and the surrounding rhizosphere soil, factors promoting the direction of evolution along the soil saprotrophy-mycorrhizal continuum mainly concern soil fungi (Selosse et al. 2018), which we also observed in the most common taxa of fungi classified as saprotrophs and root-associated (*Achaeorhizomyces*, *Mortierella*, *Umbelopsis*) or root endohytes (*Oidiodendron*). An ambiguous nutritional mode and being considered symbiotic or saprobic may also depend on the nutritional conditions of the host environment (Fernando and Currah 1996). However, fungi with an ambiguous lifestyles represent less than 3% of the sequences in our studies.

Mixture vs. pure tree zone in response to rewatering

In the three different root zones, soil fungal communities responded differently to rewatering. In the Mix root zone, we found less fluctuation in the abundances of fungal functional groups (SAPf vs ECMf) in rewatered compared to control plots throughout the time course, compared to the monospecific, spruce and beech, root zones, and we hypothesized that a positive effect of the Mix root zone would manifest in a faster resemblance to controls. There was also less fluctuation in soil fungal communities after rewatering in mixed compared to monospecific root zones, suggesting a higher resistance to drought and rewatering in the Mix zone. This is in line with a microcosm experiment, where tree mixtures (with presumed higher niche complementarity in the soil compared to monocultures) not only alleviated drought stress perceived by soil fungal communities but also reduced community fluctuations after rewatering (Gillespie et al. 2020). In fact, higher overstory tree species diversity (up to three species) is more likely to promote soil microbial diversity through indirect interactions with plant characteristics that alter soil characteristics, such as litter, than through tree species diversity per se (Thoms et al. 2010). However, in addition to the physicochemical properties of leaf litter from different plant species that lead to significant differences in microbial community composition (i.e., conifer litter is typically more recalcitrant than broadleaf litter (Setiawan et al. 2016)), root traits, including root biomass and necromass, are also important (Thoms et al. 2010). Similar to our study, trends of differentiation of the soil fungal community in beech and spruce monocultures and mixed stands were found by Likulunga et al. (2021) and explained by soil conditions and the relative abundance of conifers. When growing in mixture, beech may be able to locate more fine roots deeper than spruce (Leuschner et al. 2004). Thus, spruce roots may have been the main factor shaping the soil fungal communities in the Mix zone, being located in the upper soil layer (Zwetsloot and Bauerle 2021). The effects of rewatering include a flush of nutrients and should lead to a rapid reaction of soil SAPf communities (Manrubia et al. 2019). We expected a faster change in SAPf diversity in the mixed zone due to a more heterogeneous soil environment compared to the monospecific zone (H2), which was not the case in the first three months. Whether this positive effect will be effective in the longer term requires further study. Griffiths and Philippot (2013) reported that faster regeneration is associated with higher physiological activity of certain taxa or higher microbial diversity and that resistant or faster regenerating taxa are more likely to occur in more diverse communities. However, in our study, this was not the case, and we did not observe a higher diversity of the soil fungal community in the mixture in comparison to the monoculture.

Functional response of the soil fungal community

In support of our third hypothesis, the structure of SAPf communities in our study correlated with soil enzyme activities. Due to the high functional redundancy of microbiota, the structure of microbial communities modified by abiotic agents can still function like control communities, allowing the buffering of the changes that occur (Allison and Martiny 2008). In our study, however, the correlation of the enzyme profile, especially in the

Journal : Medium 11104 | Article No : 6564 | Pages : 22 | MS Code : 6564 | Dispatch : 23-2-2024

rewatered variant associated with altered SAPf, persisted beyond the three months of the experiment. This indicates that three months was too short a period to restore the whole soil–plant system, including the functioning of the fungal communities, to the conditions before the drought period. Responsiveness of fungal taxa to the root zone and drought legacy was associated with the highest diversity of SAPf in the spruce root zone, but the abundance of the most common taxa did not respond to rewatering in contrast to the beech and Mix root zones. In all (spruce, beech and mixed between species) root zones, the most abundant genera were mostly positively associated with hydrolytic enzyme activities (xyl, gls, glr, cbh) and negatively associated with oxidative enzyme activities (i.e., laccase). These enzymes (xyl, gls, glr, cbh) were also more active in rewatered samples in the Mix and beech root zones than in control. In the beech root zone, enzymes associated with simple carbon source use responded fast and later on were replaced by those with more recalcitrant substrates. These enzymatic changes were associated with faster changes in litter saprotrophs in the beech zone. In contrast, the dynamics of SAPf were not as pronounced in the spruce zone with phenol-rich leaf litter, suggesting that litter degraders within SAPf taxa had the ability to degrade recalcitrant materials such as lignin and cellulose (Tunlid et al. 2016). Surprisingly, we found no association between the dominant SAPf genera and laccase activity, although other genera, even those of ECMf, were positively associated with the activity of this enzyme. An earlier study by Nickel et al. (2018) at the same site showed a decrease in ECMf-associated laccase activity on roots under prolonged (3 years) drought. Russulales, a ubiquitous order of ECMf (Looney et al. 2018), are particularly common in temperate beech stands (Pena et al. 2017) but less common in spruce forests (Asplund et al. 2019). In this study, the most abundant ECMf genus was *Russula* (more abundant in the beech and Mix root zones), which was positively associated with laccase activity in all root zones. Russulales appear to be specialized for acquiring ammonium (Nygren et al. 2008) and are known to have retained lignolytic enzymes (Looney et al. 2018). This suggests that ECMf with the capability to mineralize nitrogen from phenol-protein complexes (Pellitier and Zak 2018) may be favored under drought conditions.

Although ECMf in our study were associated with changes in soil enzyme activities, more genera of SAPf were involved, and dynamics in the composition of SAPf were correlated more strongly with the enzyme profile in the rewatering, as shown by the Mantel test. ECMf and SAPf have overlapping fundamental niches (Fernandez and Kennedy 2016), but ECMf may limit the realized niche of saprotrophs and suppress their decomposer activity (Fernandez and Kennedy 2016). Our findings of a stronger positive effect of SAPf on enzyme activity in the rewatered plots indicate that in our experimental setup during the rewatering decomposition process is not slowed down by ECMf competition.

Our study of soil fungal communities was based on DNA analysis, which represents a total microbial community pool including living, dead and resting microorganisms (Lennon and Jones 2011). In a simultaneous study performed on roots, we found good agreement between RNA- and DNA-based fungal communities in the rewatering phase (Danzberger et al. 2023). This indicates that the lack of strong dynamics in our study does not reflect a methodological bias toward resting stages or DNA from dead fungi, although a certain share cannot entirely be ruled out.

Conclusion

This study highlighted that a previous drought regime over 5 years in a beech/spruce forest had an important structuring influence on soil fungal communities during the first three months after rewatering. The close relationship between the SAPf and the rapidly changing soil conditions was emphasized by the faster and stronger response of the SAPf compared to the ECMf communities. The correlation between changes in SAPf community structure and soil enzyme activities in rewatered plots also supports this conclusion. SAPf community structure may be shaped more by the type of leaf litter accessible after water contact with the substrate than by the abiotic soil condition itself, since SAPf community structure was more related to the dynamics of nitrogen levels than to soil water content and different SAPf community responses in beech vs. spruce monoculture. SAPf community

Journal : Medium 11104 | Article No : 6564 | Pages : 22 | MS Code : 6564 | Dispatch : 23-2-2024

structure varied less in the mixed tree area, suggesting a buffering mixture effect.

Our findings underline that a short period of restoration of water conditions may not allow the soil ecosystem to recover from drought. Long-term effects on soil fungal communities and their functions need to be addressed in future studies to improve our ability to predict the impacts of extreme precipitation changes on European forest soils.

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Author's contributions FW und KP designed the experiment. FW, JD and JM processed the samples, analysed and interpreted the data with support from RW and KP. JM wrote the first draft of manuscript. All the authors substantially revised the manuscript.

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Data availability The datasets generated and analyzed during the current study are available in the Sequence Read Archive (SRA) repository in the NCBI. (BioProject PRJNA994002 http://www.ncbi.nlm.nih.gov/bioproject/994002).

Declarations

Conflict of interest The authors declare no conflict of interest.

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